Title: Jak2^{V617F} Reversible Activation Shows an Essential Requirement for Jak2^{V617F} in Myeloproliferative Neoplasms

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ABSTRACT (233 words)

Janus kinases (JAKs) mediate cytokine signaling, cell growth and hematopoietic differentiation.\(^1\) Gain-of-function mutations activating JAK2 signaling are seen in the majority of myeloproliferative neoplasm (MPN) patients, most commonly due to the \(JAK2^{V617F}\) driver allele.\(^2\) While clinically-approved JAK inhibitors improve symptoms and outcomes in MPNs, remissions are rare, and mutant allele burden does not substantively change with chronic JAK inhibitor therapy in most patients.\(^3,4\) This has been postulated to be due to incomplete dependence on constitutive JAK/STAT signaling, alternative signaling pathways, and/or the presence of cooperating disease alleles;\(^5\) however we hypothesize this is due to the inability of current JAK inhibitors to potently and specifically abrogate mutant JAK2 signaling. We therefore developed a conditionally inducible mouse model allowing for sequential activation, and then inactivation, of \(Jak2^{V617F}\) from its endogenous locus using a Dre-\(rox/Cre-lox\) dual orthogonal recombinase system. Deletion of oncogenic \(Jak2^{V617F}\) abrogates the MPN disease phenotype, induces mutant-specific cell loss including in hematopoietic stem/progenitor cells, and extends overall survival to an extent not observed with pharmacologic JAK inhibition. Furthermore, reversal of \(Jak2^{V617F}\) in MPN cells with antecedent loss of \(Tet2^{6,7}\) abrogates the MPN phenotype and inhibits mutant stem cell persistence suggesting cooperating epigenetic-modifying alleles do not alter dependence on mutant JAK/STAT signaling. Our results suggest that mutant-specific inhibition of \(JAK2^{V617F}\) represents the best therapeutic approach for \(JAK2^{V617F}\)-mutant MPN and demonstrate the therapeutic relevance of a dual-recombinase system to assess mutant-specific oncogenic dependencies \textit{in vivo}.
Somatic mutations which constitutively activate JAK2 signaling are seen in the majority of MPN patients, most commonly the recurrent JAK2V617F alteration, and murine models suggest a critical role for JAK/STAT pathway mutations in promoting the MPN phenotype in vivo. In contrast to ABL1 kinase inhibition in BCR-ABL1-driven chronic myelogenous leukemia, current JAK inhibitors fail to reduce mutant clonal fraction, do not induce pathologic regression of key disease features including myeloproliferation and bone marrow fibrosis, and most patients lose their response over time. To date, second-site JAK2 mutations have not been observed as a mechanism of acquired resistance, and different mechanisms have been postulated to mediate the inadequate efficacy of JAK inhibition, including incomplete dependency on JAK2 signaling and the presence of co-occurring mutant disease alleles. We hypothesized that the limited potency of JAK inhibition relates to insufficient mutant kinase inhibition at achievable therapeutic doses, and we and others have elucidated mechanisms by which mutant JAK2 can signal in the presence of type I JAK inhibitors. However, no study to date has shown that potent, specific inhibition of mutant JAK2 can induce disease regressions, and current model systems do not accurately recapitulate reversal of endogenous mutant expression or allow assessment of oncogenic dependency in the clonal evolution to myeloid transformation. Given this, we developed a system which would definitively assess JAK2V617F dependency in MPN.

A conditional knock-in, knock-out model of Jak2V617F MPN

To assess dependency of MPN maintenance on JakV617F oncogenic signaling, we generated a Dre-rox, Cre-lox dual-recombinase Jak2V617F knock-in/knock-out mouse model (Jak2^Rox/Lox/Jak2^{RL}) by gene targeting in mouse embryonic stem cells (Fig. 1a). The close proximity of the lox sites (82 base pairs) prevents Cre-mediated deletion prior to Dre-mediated recombination and Jak2V617F induction. Once the mutant allele is activated, the lox sites separate allowing for subsequent Cre-mediated deletion of Jak2V617F, including in models where cooperating alleles are induced by antecedent Cre-mediated activation/deletion. A similar strategy, which we have termed GOLDI-Lox for governing oncogenic loci by Dre inversion and lox deletion (GOLDI-Lox), was used to target Flt3^{ITD} in the accompanying manuscript (see Bowman, R. et al. 2022). Sequencing of the
Jak2RL locus on sorted Cre reporter cells\textsuperscript{21} after Cre recombinase exposure confirmed retention of the non-recombined Jak2RL locus (Extended Data Fig. 1a). We used mRNA electroporation of Dre mRNA \textit{ex vivo} to transiently express the Dre recombinase in primary hematopoietic stem/progenitor cells (HSPCs), which efficiently induces Jak2\textsuperscript{V617F} activation and separation of lox sites by inversion (Extended Data Fig. 1b-c). By three weeks post-transplant, lethally irradiated mice transplanted with Dre-inducible Jak2RL knock-in bone marrow developed a highly penetrant and fully transplantable MPN characterized by leukocytosis with myeloid preponderance, elevated hematocrit with erythroid progenitor expansion in bone marrow, hepatosplenomegaly, and megakaryocytic hyperplasia consistent with prior Jak2\textsuperscript{V617F} conditional knock-in mouse models of MPN (Extended Data Fig. 1d-h).\textsuperscript{9} Progressive bone marrow fibrosis was also observed at 24 weeks in 9 of 15 Jak2RL knock-in mice.

To assess the reversibility of the Jak2RL construct, we cultured Dre-electroporated, lineage-negative, tamoxifen-inducible Ubc:CreER-Jak2RL cells isolated from donor mice with active MPN \textit{ex vivo} with increasing doses of 4-hydroxy-tamoxifen (4-OHT) over bone marrow endothelial cells (BMECs) (Extended Data Fig. 2a).\textsuperscript{22} Treatment with 4-OHT resulted in deletion of the Jak2\textsuperscript{V617F} allele, which was confirmed by excision polymerase chain reaction (PCR) (Extended Data Fig. 2b). Loss of Jak2\textsuperscript{V617F} significantly reduced cell numbers \textit{ex vivo} (mean 0.18 × 10\textsuperscript{6}/mL vs. 2.19 × 10\textsuperscript{6}/mL, \( p \leq 0.0001 \)), including within immunophenotypically-defined HSPC compartments, a phenotypic change not observed with vehicle-treated Jak2RL, Cre-inducible Jak2\textsuperscript{V617F} (Jak2\textsuperscript{Crelox}, \( p \leq 0.228 \)),\textsuperscript{8} or Cre-inducible wild-type (WT) cells (\( p \leq 0.114 \)) (Extended Data Fig. 2c-g). Loss of Jak2\textsuperscript{V617F} also abrogated erythropoietin-independent erythroid differentiation\textsuperscript{23} \textit{in vitro} (\( p \leq 0.01 \)), an effect not observed when cells were exposed to the JAK inhibitor ruxolitinib\textsuperscript{24} (Extended Data Fig. 2h). The cell loss observed was associated with enhanced apoptosis, which was most apparent in Mac1\textsuperscript{+} mature myeloid cells (mean 35% vs. 9.3%, \( p \leq 0.005 \)) (Extended Data Fig. 2i).

We next evaluated the impact of reversible Jak2\textsuperscript{V617F} expression \textit{in vivo}. Twelve weeks post-transplant, secondary recipient mice transplanted with Dre-electroporated Ubc:CreER-Jak2RL whole bone marrow and exhibiting MPN were administered tamoxifen (TAM) to delete Jak2\textsuperscript{V617F} (Extended Data Fig. 3a). A sequential dual recombinase reporter system\textsuperscript{25} was used to validate
Jak2V617F deletion within Cd45.2 reporter-positive cell populations (Extended Data Fig. 3b). Deletion of Jak2V617F was also validated in vivo at the transcriptional level (p ≤ 0.0001) (Extended Data Fig. 3c) and was associated with loss of constitutive JAK/STAT signaling (Fig. 1b). Consistent with our in vitro data, we observed normalization of white blood cell (WBC; mean 6.18 K/μL vs. 17.5 K/μL, p ≤ 0.0001), hematocrit (Hct; mean 52.6% vs. 79.9%, p ≤ 0.01), and platelet (mean 786 K/μL vs. 2146 K/μL, p ≤ 0.0004) parameters within 4 weeks following tamoxifen treatment that persisted until timed sacrifice at 24 weeks (Fig. 1c, Extended Data Fig. 3d). Two of 12 mice demonstrated reemergence/persistence of the MPN phenotype, both of which showed incomplete excision of the Jak2RL allele highlighting the necessity of Jak2V617F in disease maintenance (Extended Data Fig. 3e). Genetic reversal of Jak2V617F significantly prolonged overall survival (median not defined vs. 187 days, p ≤ 0.0012) and led to loss of disease-defining MPN features in the majority of mice (9/12) (Fig. 1d). Spleen weights (mean 108.9 mg vs. 542.7 mg, p ≤ 0.0001) and inflammatory cytokine levels were also reduced to normal levels in mice with Jak2V617F reversal (Fig. 1e-f). Histopathologic analysis revealed reductions in megakaryocytic hyperplasia, splenic infiltration, and absence of bone marrow and spleen fibrosis in 8 of 9 assayed Jak2V617F-deleted mice that persisted until timed sacrifice at 24 weeks (Fig. 1g, Extended Data Fig 3f). The phenotypes observed with Ubc:CreER-Jak2V617F deletion in vivo were not observed with tamoxifen administration in the absence of Jak2V617F reversal (Extended Data Fig. 4). We conclude that the MPN phenotype requires maintenance of oncogenic signaling through Jak2V617F.

Jak2V617F reversal impairs the fitness of MPN cells, including MPN stem cells

We next evaluated Dre-electroporated Jak2RL bone marrow from Cd45.2 MPN donors in competition with Cd45.1 competitor cells to explore effects of Jak2V617F deletion on peripheral blood and bone marrow mutant cell fitness (Extended Data Fig. 5a). We observed abrupt, durable reductions in Cd45.2 mutant cell fraction in the peripheral blood (mean 24.5% vs. 63.9%, p ≤ 0.001) with Jak2V617F reversion that coincided with normalization of hematologic parameters and persisted until time of sacrifice, either with early (3 weeks) or late (12 weeks) post-transplant administration of tamoxifen (Fig. 2a, Extended Data Fig. 5b). Consistent with the in vitro data, this effect was most pronounced in Mac1+ myeloid cell fractions (p ≤ 0.0001) (Extended Data Fig. 5c). In bone marrow, the reduction in mutant cell fraction among different HSPC
compartments was more pronounced, including within megakaryocytic-erythroid progenitor (MEP; Lineage-cKit\(^+\)Sca1\(^-\)Cd34\(^+\)Fcg\(^-\); \(p \leq 0.0001\)) and granulocytic-monocytic progenitor (GMP; Lineage-cKit\(^+\)Sca1\(^-\)Cd34\(^+\)Fcg\(^+\); \(p \leq 0.0001\)) populations and down to the level of the LSK (Lineage- Sca1\(^-\)cKit\(^+\); \(p \leq 0.0096\)) stem cell compartment, including primitive long-term hematopoietic stem cells (LT-HSCs; Lineage\(^-\)Sca1\(^+\)cKit\(^+\)Cd150\(^+\)Cd48\(^+\)) \((\text{Fig. 2b, Extended Data Fig. 5d-e}).\)

Recurrent MPN, as was seen in the non-competitive setting, was observed in 3 of 14 mice across both early- and late- treatment arms and corresponded with residual mutant Jak2\(^{V617F}\) expression and sustained mutant chimerism in remaining mice at sacrifice. Transplant of unfractionated Jak2\(^{RL}\)-deleted bone marrow failed to form phenotypic disease in 4 of 5 secondary transplant recipient mice consistent with depletion of disease-propagating MPN stem cells \((\text{Extended Data Fig. 5f-h}).\)

We sought to characterize the transcriptional changes seen when Jak2\(^{V617F}\) is acutely reversed. We performed RNA sequencing (RNA-Seq) analysis of purified HSPCs 3 and 7 days following Jak2\(^{V617F}\) deletion \((n = 3-4)\) compared to MPN controls \((n = 3-4)\). Transcriptional analysis of sorted, Jak2\(^{V617F}\)-deleted LSK and MEP populations revealed near-complete loss of expression of STAT5 target genes as early as 3 days post-deletion \((\text{LSK: NES } -1.77, FDR \leq 0.002; \text{ MEP: NES } -1.53, FDR \leq 0.0065)\) indicating immediate disengagement from disease-defining pathway signaling \((\text{Extended Data Fig. 6a}).\) By 7 days, we observed significant negative enrichment in IFN\(\gamma\) \((\text{NES } -1.61, FDR \leq 0.0005)\), TGF\(\beta\) \((\text{NES } -1.45, FDR \leq 0.071)\), and TNF\(\alpha\) via NF\(\kappa\)B \((\text{NES } -1.54, FDR \leq 0.0017)\) Hallmark pro-inflammatory response pathways, as well as down-regulation of MAPK \((\text{NES } -1.52, FDR \leq 0.0052)\) and MTORC1 \((\text{NES } -1.46, FDR \leq 0.0071)\) targets in LSKs suggesting abrupt reduction in pro-inflammatory and proliferative signaling in the setting of Jak2\(^{V617F}\) deletion \((\text{Fig. 2c, Extended Data Fig. 6b, Supplemental Table 1}).\) A flux towards increased expression of myeloid genes sets compared to erythroid gene sets was also observed, characterized by increased \(S100a8, S100a9, Mpo,\) and \(Hdc\) expression in LSKs, increases in GMP \((\text{mean } 14.5\% \text{ vs. } 7.8\%, p \leq 0.018)\) vs. MEP \((\text{mean } 13.8\% \text{ vs. } 32\%, p \leq 0.0025)\) frequencies within the HSPC compartment, and enrichment in bone marrow Mac1\(^+\) myeloid cells \((\text{mean } 41.7\% \text{ vs. } 27.8\%, p \leq 0.0084)\) \((\text{Fig. 2d-e, Extended Data Fig. 6c}).\) In line with reduced erythroid output, we also observed a marked decrease in heme metabolism in MEPs \((\text{NES } -2.07, FDR \leq 4.71 \times 10^{-5})\) with associated reductions in critical erythroid/megakaryocytic transcription factors and signaling
mediators, including Nfe3,26,27 Plek2,28 and Epor29 which coincided with concomitant reductions in total erythroid progenitor cell numbers (p ≤ 0.021) and significantly reduced burst forming unit-erythroid (BFU-E) colony output of Jak2V617F deleted cells (p ≤ 0.001) (Fig. 2f, Extended Data Fig. 6d-f). Assay for Transposase Accessible Chromatin with high-throughput sequencing (ATAC-seq) on Jak2V617F-deleted cKit+ cells demonstrated an increase in open chromatin with Cebp motifs (p ≤ 1x10^-10) and reduced accessibility at Gata motifs (p ≤ 1x10^-620), including at critical erythroid loci (e.g. EpoR; log2FC 1.49, FDR ≤ 0.00135), further consistent with an erythroid to myeloid lineage switch (Fig. 2g, Extended Data Fig. 6g, Supplemental Table 2).

While reduced accessibility at putative Gata target sites was observed, we did not observe differential expression of either Gata1 (p ≤ 1.0) or Gata2 (p ≤ 0.82) in Jak2V617F-deleted LSKs or MEPs compared to controls. These data suggest the transcriptional networks regulating the MPN phenotype are not obligately achieved through transcription factor expression dysregulation but through differential transcription factor-mediated output.

**Differential efficacy of Jak2V617F deletion compared to JAK inhibitor therapy**

Given the limited ability of current JAK inhibitors to achieve disease modification and/or clonal remissions in polycythemia vera and myelofibrosis, we next compared the phenotypic and transcriptional effects of JAK inhibitor therapy with ruxolitinib to the effects of Jak2V617F reversal. We first performed RNA-seq on Jak2V617F-mutant LSKs and MEPs following 7 days of ruxolitinib treatment (n = 3) and compared this to the effects of Jak2V617F deletion (n = 3). Analysis revealed that JAK-STAT target gene expression and erythroid pathway gene expression were much less potently inhibited with ruxolitinib than with Jak2V617F deletion. Specifically, Jak2V617F deletion resulted in a significant reduction in expression of negative regulators of JAK/STAT signaling (NES -1.51, p ≤ 0.003) including Socs2,30 Pim2,31 and Cish32 (Fig. 3a, Extended Data Fig. 7a, Supplemental Table 3). Meanwhile, ruxolitinib treatment was associated with a muted reduction in the same targets (Extended Data Fig. 7a), with no significant changes in STAT5 target gene expression identified by GSEA (NES -0.913, p = 0.84). Furthermore, the alterations in erythroid pathway gene expression (NES 1.45, p ≤ 0.012 vs. NES -1.82, p ≤ 0.0005) and skewing of myeloid/erythroid progenitor frequencies observed with Jak2V617F deletion were not observed with ruxolitinib (mean GMP: vehicle [VEH] 6.93% vs. ruxolitinib [RUX] 6.66% vs. TAM 20.1%, p =
0.91 vs. \( p \leq 0.00001 \), MEP: VEH 27.1% vs. RUX 35.3% vs. TAM 14.2%, \( p = 0.25 \) vs. \( p = 0.014 \)

**(Fig. 3b-c, Extended Data Fig. 7b).** Expression of the gene sets associated with TGF\( \beta \) \( ( p = 0.65 \) and TNF\( \alpha \)/NF\( \kappa \)B \( ( p = 0.90 \) inflammatory signaling pathways were also not significantly altered with ruxolitinib treatment, in contrast to \( Jak2^{V617F} \) deletion. Consistent with this lack of change, genotype-aware single-cell ATAC-Seq (scATAC-Seq) on myelofibrosis (MF) patient samples demonstrated unaltered NF\( \kappa \)B accessibility in \( JAK2^{V617F} \)-mutant HSPCs following JAK inhibitor treatment (**Fig. 3d, Extended Data Fig. 7c**; see Myers, R. and Izzo, F. *et al.*, bioRxiv, 2022) supporting the notion of insufficient mitigation of inflammatory signaling by JAK inhibition on MPN-sustaining stem cells.

To evaluate the phenotypic effects of \( Jak2^{V617F} \) deletion in direct comparison to JAK kinase inhibition, we performed an *in vivo* trial lasting 6 weeks comparing ruxolitinib to \( Jak2^{V617F} \) deletion (**Extended Data Fig. 7d**). We saw a greater improvement in hematologic parameters, spleen weights (mean VEH 457 mg vs. RUX 235 mg vs. TAM 125 mg, \( p \leq 0.0027 \)), restoration of histopathologic morphology in both bone marrow and spleen, and reduced Cd45.2 mutant chimerism in peripheral blood (mean VEH 40.7% vs. RUX 37.7% vs. TAM 17.3%, \( p \leq 0.0059 \)) of \( Jak2^{V617F} \)-deleted mice versus ruxolitinib treated mice (**Fig. 3f, Extended Data Fig. 7e-g**). Most importantly, the reduction in mutant cell fraction seen with \( Jak2^{V617F} \) deletion within hematopoietic progenitor (GMP: \( p \leq 0.0001 \), MEP: \( p \leq 0.0001 \)) and LSK stem cell enriched populations (mean VEH 87.9% vs. RUX 87.6% vs. TAM 28.7%, \( p \leq 0.0001 \)) was not observed with pharmacologic JAK inhibition (**Fig. 3g**). These data indicate that current JAK inhibitors do not sufficiently inhibit mutant JAK2 signaling and that more potent target inhibition offers the potential for greater therapeutic efficacy.

Previous studies have suggested that MAPK signaling plays an important role in MPN disease cell survival in the setting of type I JAK inhibitor therapy,\(^{17} \) and recent work has implicated the MAPK-dependent factor YBX1 as a critical mediator of \( JAK2^{V617F} \)-mutant cell persistence.\(^{18} \) We observed distinct effects on MAPK activity by RNA-Seq with ruxolitinib treatment vs. \( Jak2^{V617F} \) deletion in comparison to vehicle treated mice. Negative regulators of KRAS signaling were down-regulated with ruxolitinib (NES -1.64, \( FDR \leq 0.0005 \)) and up-regulated with \( Jak2^{V617F} \) deletion (NES 1.35, \( FDR \leq 0.039 \)) suggesting enhanced MAPK signaling with ruxolitinib and MAPK attenuation with
Jak2V617F deletion (Fig. 3h). Immunohistochemistry of bone marrow sections confirmed increased phospho-ERK abundance in ruxolitinib-treated mice that was abrogated with Jak2V617F deletion (Fig. 3i), and genotype-specific scATAC-seq revealed increased accessibility of MAPK-mediated AP-1 factors FOS/JUN33 within HSPCs of ruxolitinib-treated MF patients in comparison to untreated MF HSPCs consistent with enhanced MAPK activity (Extended Data Fig. 7h). Furthermore, expression of Ybx1 in sorted murine cKit+ cells was increased with ruxolitinib therapy but potently suppressed with Jak2V617F deletion (mean rel. exp. VEH 1.37 vs. RUX 2.52 vs. TAM 0.42, p ≤ 0.0094) (Fig. 3j). These data suggest that potent, mutant-specific Jak2V617F targeting can abrogate pathologic MAPK signaling and Ybx1-mediated persistence of Jak2V617F-mutant HSPCs.

Jak2V617F dependency with cooperative Tet2 loss

Previous studies of mutational order in primary MPN cells have shown that cooperating mutations in epigenetic regulators, including TET2, can precede the acquisition of JAK2V617F in the clonal evolution of MPN and that antecedent TET2 mutations can alter the in vitro sensitivity to ruxolitinib. In addition, in vitro and in vivo studies have shown that concurrent TET2 and JAK2V617F mutations promote enhanced mutant hematopoietic stem cell (HSC) fitness and increased risk of disease progression.35-37 Our Jak2RL system allows for the assessment of Jak2V617F dependency in the setting of co-occurring mutant allele activation/inactivation, including in the context of antecedent mutations in epigenetic regulators. We therefore assessed the impact of Jak2V617F activation in concert with pre-existing Tet2 loss with the reversible Jak2RL allele (Fig. 4a). Mice transplanted with Dre-electroporated Ubc:CreER-Jak2RL/Tet2Δ cells demonstrated enhanced leukocytosis (mean 27.0 K/μL vs. 21.0 K/μL, p ≤ 0.021), increased spleen volumes (mean 626 mg vs. 374.8 mg, p ≤ 0.0047), expanded mutant peripheral blood chimerism (mean 61.9% vs. 24.9%, p ≤ 0.0042), and improved serial replating capacity in colony forming assays compared to Ubc:CreER-Jak2RL transplanted mice (Fig. 4b-d, Extended Fig. 8a-b). These data are consistent with previous Jak2V617F/Tet2Δ models35,36 and highlight the utility of the Dre-Cre dual recombinase system to model evolution from pre-malignant, clonally restricted hematopoietic states (i.e. clonal hematopoiesis [CH]) to overt MPN. Ex vivo co-culture of Jak2RL/Tet2Δ cells over BMECs exhibited a near 2-fold increase in hematopoietic cell output (mean 2.32 × 10⁶/mL vs.
1.18 × 10^6/mL, \( p \leq 0.0017 \)), particularly among Mac1^+ mature myeloid cells (mean 0.69 × 10^6/mL vs. 0.35 × 10^6/mL, \( p \leq 0.011 \)), compared to \( \text{Jak}2^{\text{RL}} \) cells consistent with the known role of Tet2 loss-of-function in enhancing myeloid lineage commitment (Extended Data Fig. 8c).^6

We next evaluated effects of \( \text{Jak}2^{V617F} \) deletion on \( \text{Jak}2^{\text{RL}}/\text{Tet}2^{-/-} \) mutant cell fitness in vivo in competition with Cd45.1 bone marrow. Treatment with tamoxifen at 9 weeks post-transplant resulted in normalization of hematologic parameters (\( p \leq 0.005 \)) and reductions in peripheral blood mutant cell fraction of double-mutant cells to a similar extent observed with \( \text{Jak}2^{V617F} \) deletion in single-mutant \( \text{Jak}2^{\text{RL}} \) transplanted mice (Fig. 4e-f). Further, spleen sizes (mean 103 mg vs. 529 mg, \( p \leq 0.0001 \)) and total BM cellularity (femur; mean 11.6 × 10^6/mL vs. 15.7 × 10^6/mL, \( p \leq 0.0035 \)) were also normalized, and while the extent of reticulin fibrosis was increased in \( \text{Jak}2^{\text{RL}}/\text{Tet}2^{-/-} \) mice compared to \( \text{Jak}2^{\text{RL}} \), mutant allele reversal resolved fibrosis in both mutational contexts (Fig. 4g, Extended Data Fig. 8d-e). The reduction in mutant cell fraction, as was observed with single-mutant mice, persisted down to the level of HSPCs in tamoxifen treated \( \text{Jak}2^{\text{RL}}/\text{Tet}2^{-/-} \) mice, including within the LSK stem cell-enriched compartment (mean 28.7% vs. 73.7%, \( p \leq 0.001 \)) (Fig. 4h, Extended Data Fig. 8f). In a subset of assayed \( \text{Jak}2^{\text{RL}}/\text{Tet}2^{-/-} \) mice following \( \text{Jak}2^{V617F} \) deletion (4/9), we were unable to detect \( \text{Tet}2^{-/-} \) knock-out bands in whole marrow at time of sacrifice, and cells harvested from \( \text{Jak}2^{\text{RL}}/\text{Tet}2^{-/-} \) recipient mice following oncogenic deletion were unable to serially replate indicating loss of self-renewal capacity in comparison to control double-mutant mice (Fig. 4i, Extended Data Fig. 8g). These data support the notion that co-occurring loss-of-function mutations of Tet2 do not dramatically alter reliance on JAK/STAT signaling for disease maintenance, and that despite the fitness advantage engendered by Tet2 loss on MPN hematopoietic stem cells, the reductions in HSC fitness in the setting of \( \text{Jak}2^{V617F} \) reversion suggest a unique dependency on oncogenic \( \text{Jak}2^{V617F} \) that renders double-mutant cells susceptible to eradication.

Conclusion

Mutated kinases occur frequently in cancer and are amenable to targeted inhibition; however, mechanisms mediating acquired resistance have been observed for most targeted therapies.\(^3\) By contrast, current JAK inhibitors fail to eliminate \( \text{JAK}2^{V617F} \)-mutant clones in MPN patients
suggesting inadequate target inhibition and/or other genetic/non-genetic factors mediate JAK2V617F-mutant cell persistence in the setting of JAK inhibitor therapy.5 We show in preclinical models that there is an absolute requirement for Jak2V617F in MPN cells and that mutant-specific targeting of Jak2V617F abrogates MPN features, reduces mutant cell fraction, and extends overall survival with concomitant depletion of disease-sustaining stem cells within the HSPC compartment. Further, our data suggest that Jak2V617F dependency persists even in the setting of antecedent mutations in epigenetic regulators, specifically Tet2. These data support the notion that improved targeting of JAK2 signaling and downstream effectors offers greater therapeutic potential than current JAK kinase inhibitors and that JAK2V617F mutant-selective inhibition represents a potential curative strategy for the treatment of MPN patients. Moreover, we demonstrate the feasibility of our dual-recombinase system to evaluate oncogenic signaling dependencies in vivo, and we believe that a similar approach will allow us to assess oncogenic dependencies and mechanisms of mutant-mediated transformation across a spectrum of malignant contexts.
METHODS

Experimental animals All animal studies were performed in accordance with institutional guidelines established by Memorial Sloan Kettering Cancer Center (MSKCC) under the Institutional Animal Care and Use Committee-approved animal protocol (#07-10-016) and the Guide for the Care and Use of Laboratory Animals (National Academy of Sciences 1996). All experimental animals were maintained on a 12 hour light-dark cycle with access to water and standard chow ad libitum. Veterinary staff provided regular monitoring and husbandry care. All mice had intact immune systems, were drug and test naïve, and had not been involved in previous procedures. Animals were monitored daily for signs of disease or morbidity, bleeding, failure to thrive, infection, or fatigue and sacrificed immediately if they exhibited any signs of the above. Mice harboring the Jak2RL allele were generated by Ingenious Targeting Laboratory (Ronkonoma, NY) in a C57BL/6 background. Specifically, a 8.86kb genomic DNA used to construct the targeting vector was first subcloned from a positively identified C57BL/6 BAC clone (RP23-316C6). The region was designed such that the long homology arm (LA) extends ~6 kb 5’ to the cluster of Lox2272-Rox-Rox12-Lox2272 sites, and the short homology arm (SA) extends about 2.2 kb 3’ to the Neo cassette and 3’ Rox12 site. The inversion cassette is in between the second set of Lox2272 and Rox sites, and it consists of the mutant exon 14* (V617F) and its flanking genomic sequences for correct splicing (SaE14*Sd). The inversion cassette replaces wild-type exon 14 and the same flanking genomic sequences included in the cassette. The BAC was sub-cloned into a ~2.4kb pSP72 (Promega) backbone vector containing an ampicillin selection cassette for retransformation of the construct. Ten micrograms of the targeting vector was then linearized and transfected by electroporation of FLP C57Bl/6 (B6) embryonic stem cells. After selection with G418 antibiotic, surviving clones were expanded for PCR analysis to identify recombinant ES clones. After successful clone identification, the neomycin cassette was removed with a transient pulse of Cre recombinase and clones were reconfirmed following expansion. Finally, ES cells were injected in C57BL/6 mice via tetraploid complementation (NYU). Tet2f/f conditional knock-out mice, Cre-lox Jak2V617F knock-in mice, RC::RLTG reporter mice, Cre TdT Tomato reporter mice, and Ubc:CreER mice have been described previously.6,8,21,25,39 6-8 week old female and male Jak2RL or Jak2RL/Tet2f/f donor mice were used for Dre electroporation knock-in experiments. Age-
matched 6-10 week old female mice were used as donors for all transplant experiments (Ly5.1 Cd45.1 competitive or C57BL/6 non-competitive).

**Mouse genotyping** DNA was isolated using the DNeasy Blood & Tissue Kit (Qiagen, Germantown, MD). Jak2^{V617F} knock-in genotyping was carried out using the following primers:

FWD: 5'-GCCATCTTTCCAGCCTAAAATTAG-3'; REV: 5'-TCCAAAGAGTGCTGTAAGTAGACAACT-3' and with the following reaction conditions: 94°C for 3 minutes followed by 15 cycles of 94°C for 15s, 65°C for 15s, and 72°C for 30s decreasing by 1°C per cycle, and then followed by an additional 25 cycles of 94°C for 15s, 50°C for 15s, and 72°C for 30s. Jak2^{V617F} knock-out genotyping was carried out using the following primers: FWD: 5'-GCCATCTTTCCAGCCTAAAATTAG-3'; REV: 5'-ACCAGTAGCTCAGGAGGTTACACG-3' and with the following reaction conditions: 94°C for 2 minutes followed by 30 cycles of 94°C for 30s, 53°C for 30s, and 72°C for 30s. Sequencing of the unrecombined Rox-lox locus was carried out using the following primers: FWD: 5'-AGGAGCATCGATGACTACATGAG-3'; REV: 5'-AGACTCTCCCACGGTGCTCATCTACG-3' and with the following reaction conditions: 98°C for 30 seconds followed by 35 cycles of 98°C for 10s, 65°C for 15s, and 72°C for 30s. Tet2 genotyping were carried out using the following primers/conditions: FWD: 5'-AAGAATTGCTACAGGCCTGC-3'; REV: 5'-TTCTTTAGCCCTTGCTGAGC-3'; ExR: 5'-TAGAGGGAGGGGCTACATGAG-3' and with the following reaction conditions: 94°C for 2 minutes followed by 39 cycles of 94°C for 35s, 58°C for 45s, and 72°C for 55s. Annotation of PCR genotyping results was carried out on a QIAxcel Advanced System (Qiagen) and analyzed using QIAxcel ScreenGel software (Qiagen). Sanger sequencing was performed by Genewiz (South Plainfield, NJ) and analyzed using Benchling software.

**Dre mRNA electroporation** Dre mRNA was purchased from TriLink Biotechnologies (San Diego, CA) and electroporation carried out using the Neon Transfection System (ThermoScientific) per the manufacturer’s protocol. Specifically, bone marrow donor cells were isolated from limb bones into phosphate buffered saline (PBS; pH 7.2) containing 2% fetal calf serum via centrifugation. After red blood cell (RBC) lysis, single-cell suspensions were depleted of lineage-committed hematopoietic cells using a Lineage Cell Depletion Kit according to manufacturer’s protocol (EasySep™, StemCell Technologies, Inc.). 2.5-3.0 × 10^6 lineage-depleted bone marrow was then
washed in PBS and then resuspended in 135 μL Buffer T to which 15 μL of Dre mRNA (at 1 μg/μL) was quickly added and electroporated at the following conditions: 1700V for 20ms x1 pulse. The cells were then pipetted into penicillin-streptomycin free StemSpan SFEM medium with thrombopoietin (TPO; 20 ng/mL; PeproTech) and stem cell factor (SCF; 20 ng/mL; PeproTech), cultured for two hours, and then subsequently harvested and washed/resuspended in PBS and transplanted via lateral tail vein injection into lethally irradiated (900cGy) 6-8 week old C57BL/6J recipient mice at approximately 4 × 10⁵ cells per recipient along with 50,000 un-electroporated wild-type whole bone marrow support cells. Double-mutant Jak2⁵RL/Tet²f/f transplants/electroporations were carried out as above, except donor mice were dosed with tamoxifen (100 mg/kg by oral gavage daily x4; purchased from MedChemExpress) 6-8 weeks prior to harvest and excision confirmed prior to Dre electroporation.

Transplantation assays and in vivo experiments. Jak2⁵RL and Jak2⁵RL/Tet²f/f lines were crossed to Ubc:CreER tamoxifen-inducible Cre lines and RLTG dual-recombinase reporter lines.₂⁵,₃⁹ Primary recipient mice transplanted with Dre mRNA-recombined Ubc:CreER-Jak2⁵RL or Ubc:CreER-Jak2⁵RL/Tet²f/f bone marrow cells were bled every 3-4 weeks post-transplant to monitor disease status. Peripheral blood was isolated by submandibular bleeds and complete blood counts determined using a ProCyte Dx (IDEXX Laboratories, Westbrook, ME) per manufacturer’s instruction. For competitive repopulation assays, 1.2 × 10⁶ whole bone marrow from primary transplant recipient mice exhibiting MPN was harvested 6-8 weeks post-transplant and combined with age-matched 0.8 × 10⁶ Cd45.1 (Jackson Laboratories, Bar Harbor, ME) whole bone marrow and transplanted into 6-8 week old lethally irradiated Cd45.1 secondary recipient mice. Mice transplanted with Dre-recombined Jak2⁵1617F cells demonstrating low Cd45.2 chimerism at baseline (<15%) and/or evidence of poor MPN cell engraftment were excluded from study cohorts. To induce Cre and delete Jak2⁵1617F, mice were treated with tamoxifen (TAM; purchased from MedChemExpress) 100 mg/kg daily (dissolved in corn oil) by oral gavage x 4 followed by 14 days of TAM chow approx. 80 mg/kg daily (ENVIGO). Tamoxifen control studies were carried out using similar dosing schedules on 45.1 mice transplanted in competition with Dre-electroporated, Cre-negative Jak2⁵RL MPN bone marrow cells. For terminal tissue isolation, mice were euthanized with CO₂ asphyxiation, and tissues were dissected and fixed with 4% paraformaldehyde for histopathological analysis. For whole bone marrow isolation, the femurs, hips, and tibias were...
dissected and cleaned. Cells were then isolated using centrifugation at 8000xG for 1 minute followed by RBC lysis (BioLegend) for 10-15 minutes. Bone marrow cell numbers and viability were determined using an automated cell counter (ViCell Blu, Beckman Coulter). Spleen cell suspensions were generated by crushing whole spleen and filtering through a 70 µM filter. RBC lysis (BioLegend) was performed and cells were prepared for downstream processing or frozen.

In vivo drug studies For in vivo inhibitor studies, approximately 8 weeks following transplant, secondary transplant cohorts of lethally-irradiated mice transplanted with Ubc:CreER-Jak2^{RL} bone marrow in competition with Cd45.1 marrow (as above) and exhibiting active MPN were bled and cohorted based on peripheral blood Cd45.2 chimerism and total WBC count to achieve congruency across treatment arms. Mice were then treated with ruxolitinib (60 mg/kg P.O. twice daily; dissolved in 20% Captisol in PBS; purchased from MedChemExpress), tamoxifen to delete Jak2^{V617F} (as above; purchased from MedChemExpress), or vehicle. Investigators were not blinded to the identity of mice or samples. Mice were treated for a total of 6 weeks before timed sacrifice and marrow/spleen harvested as above.

Bone marrow endothelial cell (BMEC) culture Bone marrow cells were isolated from limb bones into FACS buffer (phosphate buffered saline [PBS] + 2% fetal bovine serum) via centrifugation. After RBC lysis, single-cell suspensions were depleted of lineage-committed hematopoietic cells using a Lineage Cell Depletion Kit according to manufacturer’s protocol (EasySep™, StemCell Technologies, Inc.). Subsequently, 50,000 of the resulting lineage- cells were plated on a confluent monolayer of BMECs in a single well of a 12-well plate. Each well had 1 mL StemSpan SFEM (StemCell Technologies, Inc.) with 20 ng/mL recombinant murine SCF (PeproTech) in addition to the corresponding drug treatment: either 4-hydroxytamoxifen (4-OHT; Sigma Aldrich; stock concentration: 13 mM) or its vehicle, appropriately diluted in media to its final concentration (i.e., 0.01% (v/v) of ethanol (EtOH), or 200 nM, 400 nM or 1 μM of 4-OHT) (three replicates/condition). The BMECs were seeded two days before plating the lineage- cells at a density of 100,000 cells/well. Co-cultures were maintained for a total of 7 days at 37°C and 5% CO₂, with media being completely refreshed with the original SCF and drug/vehicle concentrations. 4-OHT or EtOH vehicle was added to the culture on day 1 and again on day 4. On day 7, total cells were harvested with Accutase (Biolegend) and cell numbers were determined via
an automatic cell counter (ViCell Blu, Beckman Coulter). Cells were then stained with the desired antibody cocktail and phenotyped by flow cytometry.

**Flow cytometry and western blot** Following single cell preparation, murine peripheral blood, whole bone marrow, or spleen mononuclear cells were lysed for 10-15 minutes with RBC lysis buffer (BioLegend, San Diego, CA) and washed twice with FACS buffer. Cells were then resuspended in Fc (Cd16/32) block for 15 minutes and then subsequently stained with a cocktail comprised of antibodies targeting Cd3 (17A2), Cd45R/B220 (RA3-6B2), Gr-1 (RB6-8C5), Cd11b (M1/70), Cd45.2 (104), and Cd45.1 (A20) for 30 minutes. For hematopoietic stem/progenitor cell analysis, lysed bone marrow was stained with a cocktail of lineage markers along with antibodies against c-Kit (2B8), Sca-1 (D7), FcγRII/III (2.4G2), Cd34 (RAM34), Cd150 (9D1), and Cd48 (HM48-1). Erythroid progenitor flow was carried out on unlysed bone marrow or spleen with the addition of the following antibodies: Cd105, Cd71 (R17217), Cd41 (MWReg30), and Ter119 (Ter-119). All FACS antibodies were purchased from BD, BioLegend, or eBioscience. Following antibody incubation, cells were washed with FACS buffer and resuspended in a DAPI-containing FACS buffer solution for analysis and sorting. Samples were run on a LSRFortessa (Becton Dickinson) using FACSDiva software and analyzed with FlowJo (Treestar, Ashland, OR, USA).

For Western blot analysis, whole-cell protein extracts from harvested splenocytes were prepared using RIPA buffer (ThermoScientific, Rockford, IL) containing a protease/phosphatase inhibitor cocktail (Thermo Scientific). Protein quantification was performed using the Pierce BCA protein assay kit (ThermoScientific) and analyzed on a Cytation 3 plate reader (BioTek). Proteins were separated by NuPAGE 4-12% Bis-Tris Gel and transferred to a nitrocellulose membrane. The following antibodies were used: β-actin (Cell Signaling 4970S), STAT5 (Cell Signaling 94205S), and pSTAT5 (Cell Signaling, 9359S). Images were obtained using the ChemiDoc Imaging System (BioRad) and analyzed using ImageLab software (BioRad).

**Histology staining and immunohistochemistry (IHC), photography** Tibia and spleen samples were fixed in 4% paraformaldehyde for over 24 hours and then embedded in paraffin. Paraffin sections were cut on a rotary microtome (Mikrom International AG), mounted on microscope slides (ThermoScientific), and air-dried in an oven at 37°C overnight. After drying, tissue section slides were processed either automatically for hematoxylin and eosin (H&E) staining (COT20
stainer, Medite), or manually for reticulin staining. All samples and slide preparation, including immunohistochemistry was carried out at the Tri-Institutional Laboratory of Comparative Pathology (LCP) core facility. The following antibodies were used for immunohistochemistry: Mac1 (Cedarlane CL8941B, 1:100), Ter119 (BDBioscience, 550565 1:200), and phospho-44/42 MAPK (Erk1/2) (Cell Signaling 4376, 1:100). Pictures were taken at 100X, 200X and 400X (H&E, reticulin and respective IHC) magnification using an Olympus microscope and analyzed with Olympus Cellsens software. Tissue sections were formally evaluated by a hematopathologist (W. Xiao), including reticulin scoring.

Assessment of apoptosis and viability Apoptosis was measured by flow cytometry on a LSRFortessa (Becton Dickinson) cytometer with Annexin V PerCPCy5.5 antibody (BioLegend) in combination with the antibody cocktail (above) in Annexin binding buffer (BioLegend) at 1:50 dilution in combination with DAPI as live/dead cell stain.

Colony forming assays To assess colony formation and serial replating capacity, RBC-lysed 50,000 whole bone marrow cells were seeded in 1.5mL MethoCult M3434 (Stem Cell Technologies) with no additional supplemental cytokines in triplicate on 6 well plates and scored on day 8. For replating, cells were harvested and pooled and then re-seeded once more at 50,000 cells/well in 1.5mL MethoCult M3434 in triplicate.

Serum cytokine profiling Serum samples were diluted two-fold with PBS (pH 7.2) and stored at -80°C until analysis. Cytokine levels were measured in duplicates (62.5μL each) by Eve Technologies Corporation (Mouse Cytokine Array/Chemokine Array 44-Plex, Calgary, AB, Canada).

RNA sequencing (RNA-Seq) and data analysis For gene expression analysis, secondary cohorts of lethally irradiated C57BL/6 mice transplanted with Ubc:CreER-Jak2RL-Jak2RLTG reporter bone marrow 8 weeks post-transplant and exhibiting MPN were treated with ruxolitinib (60mg/kg P.O. twice daily), tamoxifen (100mg/kg by oral gavage daily × 4 followed by 80mg/kg of TAM chow × 3 days) +/- vehicle (MPN control) for 7 days and then sacrificed. Lineage-depleted bone marrow was isolated and stained with an antibody cocktail containing a combination of lineage markers
along with antibodies against c-Kit (2B8), Sca-1 (D7), FcγRII/III (2.4G2), and Cd34 (RAM34) for
30 minutes, washed, and then resuspended in FACS buffer containing DAPI as a live/dead stain.

TdTomato+ (Jak2<sup>RL</sup> knock-in) or GFP+ (Jak2<sup>RL</sup> knock-out) LSKs and MEPs were then sorted on
a FACSaria III directly into Trizol LS (Invitrogen) and stored at -80°C until processing. RNA was
subsequently isolated using the Direct-Zol Microprep Kit (Zymo Research, R2061) according to
manufacturer’s protocol and quantified using the Agilent High Sensitivity RNA ScreenTape
(Agilent 5067-5579) on an Agilent 2200 TapeStation. cDNA was generated from 1 ng of input
RNA using the SMART-Seq HT Kit (Takara 634455) at half reaction volume followed by Nextera
XT (Illumina FC-131-1024) library preparation. cDNA and tagmented libraries were quantified
using High Sensitivity D5000 ScreenTape (5067-5592) and High Sensitivity D1000 ScreenTape
respectively (5067-5584). Libraries were sequenced on a NovaSeq at the Integrated Genomics
Operation (IGO) at MSKCC. FASTQ files were mapped and transcript counts were enumerated
using STAR (genome version mm10 and transcript version ensembl M13). Counts were input into
R and RNA-sequencing analysis using DESeq2. Genes were filtered out prior to modeling in
DESeq if they were not detected in all, with MEPs and LSKs modeled separately. Differentially
expressed genes were identified with a log2-foldchange of 1 and an adjusted <i>p</i> value of 0.05. Gene
set enrichment analysis was performed using the fgsea package at 100,000 permutations with
genesets extracted from the msigdbr package. Single sample gene set enrichment analysis was
performed using the gsva package. Figures were generated using ggplot2 and tidyheatmaps
packages. Complete scripts can be found on github at https://github.com/bowmanr/goldilox.

**Mouse Assay for Transposase-Accessible Chromatin Sequencing (ATAC-Seq) and data analysis**

Chromatin accessibility assays utilizing the bacterial Tn5 transposase were performed as
described. Briefly, 5.0 × 10<sup>4</sup> TdTomato+ (Jak2<sup>RL</sup> knock-in) or GFP+ (Jak2<sup>RL</sup> knock-out) cKit<sup>+</sup>
bone marrow cells from mice treated for 7 days with tamoxifen or an untreated MPN control cohort
were sorted on a FACSaria III directly into PBS and subsequently lysed and incubated with
transposition reaction mix containing PBS, Tagment DNA buffer, 1% Digitonin, 10% Tween-20,
and Transposase (Illumina). Samples were then incubated for 30 minutes at 37°C in a thermomixer
at 1000 rpm. Prior to amplification, samples were concentrated with the DNA Clean and
Concentrator Kit-5 (Zymo). Samples were eluted in 20 μL of elution buffer and PCR-amplified
using the NEBNext 2X Master Mix (NEB) for 11 cycles and sequenced on a NextSeq 500
(Illumina). All samples were processed at the Center for Epigenetics Research (CER) core facility at MSKCC. Libraries were sequenced on a NovaSeq at the Integrated Genomics Operation (IGO) at MSKCC. Data analysis was completed through in house scripts at the CER, in brief: reads were trimmed with ‘trim_galore’ and aligned to mouse genome mm9 using bowtie2 (default parameters). Duplicates were removed with the Picard tool ‘MarkDuplicates’, and peaks were called with MACS2, merged and used to create a full peak atlas. Read counts were tabulated over this atlas using featureCounts. Downstream differential enrichment testing was completed in DESeq2 with default normalization scheme. HOMER was used for known motif enrichment amongst the differentially enriched peaks as defined by a fold change of +/- 1.5 and an adjusted p value of 0.1.

**Human single-cell ATAC-Seq and data analysis** Single-cell ATAC-seq data was processed using cellranger-ATAC (v2.0.0) mkfastq. ATAC sequencing reads were then aligned to the hg38 reference genome using cellranger-ATAC count function. Fragment files generated by cellranger-ATAC were used as input for the ArchR⁴¹ (v1.0.0). For initial dimensionality reduction and patient data integration, the cell by genomic bin matrix was used as input for reciprocal latent semantic indexing (LSI) as calculated by the Signac (v1.1.1). Transcription factor motif accessibility z-scores were calculated with ChromVAR⁴² (v1.8.0). The earliest HSPCs (cluster HSPC1, see Myers, R. and Izzo, F. *et al*., bioRxiv, 2022) were subset for downstream analysis, and statistical comparisons of motif accessibility for NFKB1, REL, FOS, and JUN transcription factors were performed via linear mixture model including patient identity as random effect to account for potential technical confounders arising from sample-specific batch effects. For heatmap representation, motif accessibility z-scores were used as input and the pheatmap (v1.0.12) R package was used.

**Quantitative real-time PCR** Total RNA was extracted from magnetic-bead isolated cKit⁺ bone marrow (Miltenyi Biotec) using the Direct-zol RNA extraction kit (Zymo) per manufacturers’ protocols respectively. Complementary DNA was then reverse transcribed using the Verso cDNA Synthesis kit (ThermoFisher Scientific). *Ybx1* expression was evaluated by quantitative reverse-transcription (qRT) PCR using Taqman probes purchased from ThermoFisher (Mm00850878_g1) on the RealPlex thermocycler (ThermoFisher Scientific, Fairlawn, NJ).
Statistical analysis Statistical analyses were performed using Student’s t-test (normal distribution) using GraphPad Prism version 6.0h (GraphPad Software, San Diego, CA) unless otherwise noted. Kaplan-Meier curves were determined using the log-rank test. \( P<0.05 \) was considered statistically significant. The number of animals, cells and experimental replication can be found in the respective figure legends.

Data availability All raw and processed sequencing data is made available at https://github.com/bowmanr/goldilox and via the NCBI Gene-Expression Omnibus (GEO).

REFERENCES

plasms.

Combinase, like Cre, is a highly efficient site

651


650

Chapeau, E. A. et al. A conditional inducible JAK2V617F transgenic mouse model reveals

myeloproliferative disease that is reversible upon switching off transgene expression. PLoS

649


648

Castagnetti, F. et al. Long-term outcome of chronic myeloid leukemia patients treated


647


646

(Blood).

645

Brkic, S. & Meyer, S. C. Challenges and Perspectives for Therapeutic Targeting of

644


643

Koppikar, P. et al. Heterodimeric JAK-STAT activation as a mechanism of persistence to

642


641

Stivala, S. et al. Targeting compensatory MEK/ERK activation increases JAK inhibitor


640

Jayavelu, A. K. et al. Splicing factor YBX1 mediates persistence of JAK2-mutated

639


638

Anastassiadis, K. et al. Dre recombinase, like Cre, is a highly efficient site-specific

637


636

Sauer, B. Inducible gene targeting in mice using the Cre/lox system. Methods 14, 381-392,


635

Madisen, L. et al. A robust and high-throughput Cre reporting and characterization system

634


633

Poulos, M. G. et al. Vascular Platform to Define Hematopoietic Stem Cell Factors and

632


631

Socolovsky, M. et al. Ineffective erythropoiesis in Stat5a(-/-)5b(-/-) mice due to decreased

630


629

Quintas-Cardama, A. et al. Preclinical characterization of the selective JAK1/2 inhibitor

628

INCB018424: therapeutic implications for the treatment of myeloproliferative neoplasms.

627


626

Plummer, N. W. et al. Expanding the power of recombinase-based labeling to uncover


625


624

Erythroid transcription factor NF-E2 is a haematopoietic-specific basic-leucine zipper

623


622

Jutzi, J. S. et al. MPN patients harbor recurrent truncating mutations in transcription factor

621


620

Zhao, B. et al. Loss of pleckstrin-2 reverts lethality and vascular occlusions in

619


618


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AUTHOR CONTRIBUTIONS

A.D., R.L.B. and R.L.L. conceived the project, designed the experiments, and analyzed the data. In vivo work was performed primarily by A.D. with technical assistance from Y.P., A.K., W.J.K., A.N, M.B, M.F, S.C., L.C., B.W., W.A., S.M., S.E., T.M.. Additional project design provided by A.V.. Hematopathology was formally interpreted by W.X.. Bone marrow endothelial experiments were performed by A.D., M.W., and I.F.M.. R.L.B., J.Y, and R.K. performed the computational analysis. Single-cell human ATAC data was performed and analyzed primarily by F.I., R.M.M, with support from D.L.. A.D., R.L.B., and R.L.L. contributed to the initial manuscript drafts. All

COMPETING INTEREST DECLARATION

R.L.L. is on the supervisory board of Qiagen and is a scientific advisor to Imago, Mission Bio, Baxx, Zentalis, Ajax, Auron, Prelude, C4 Therapeutics and Isoplexis. He has received research support from Abbvie, Constellation, Ajax, Zentalis and Prelude. He has received research support from and consulted for Celgene and Roche and has consulted for Syndax, Incyte, Janssen, Astellas, Morphosys and Novartis. He has received honoraria from Astra Zeneca and Novartis for invited lectures and from Gilead and Novartis for grant reviews. D.A.L. has served as a consultant for Abbvie and Illumina and is on the Scientific Advisory Board of Mission Bio and C2i Genomics. D.A.L. has received prior research funding from BMS, 10X Genomics and Illumina unrelated to the current manuscript. S.F.C. is a consultant for and holds equity interest in Imago Biosciences. R.L.B. has received honoraria from Mission Bio and is a member of the Speakers Bureau for Mission Bio. No other authors report competing interests.

MATERIALS AND CORRESPONDENCE

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Figure 1

a) Diagram showing the genetic modifications and experimental setup.

b) Western blot analysis showing the expression of pSTAT5, tSTAT5, and β-Actin under different conditions.

c) Graphs showing the change in WBC and Hematocrit over time.

d) Graph showing the survival probability over time.

e) Graph showing the spleen weight over time.

f) Heatmap representing gene expression differences between WT, CTRL, and TAM conditions.

g) Histological images comparing H&E and Reticulin staining between Control and Tamoxifen-treated samples.
Figure 1: Jak2V617F deletion abolishes JAK/STAT signaling and abrogates the MPN phenotype. 

a. Schematic representation of the dual-recombinase Jak2V617F conditional knock-in/knock-out allele (Jak2RL), the Jak2RL knock-in allele following Dre recombination, and the null recombined allele following Cre-mediated deletion. Semi-circles indicate Rox sequences; triangles indicate loxP sequences. 

b. Representative western blot depicting phospho-STAT5 abundance of Dre-mediated Jak2V617F knock-in (+Dre) vs. Jak2V617F-deleted (+Dre +Cre) states from isolated splenocytes 7 days following tamoxifen administration in comparison to unrecombined (Unrec.) Jak2RL cells (n = 2 biological replicates each; representative of n = 2 independent experiments). 

c. Peripheral blood count trends (weeks 0-24) of MPN (CTRL) vs. tamoxifen (TAM; Jak2V617F-deleted) treated mice: white blood cells (WBC; left panel), hematocrit (Hct; right panel) (n ≥ 10 per arm; mean ± s.e.m). Gray bar represents duration of TAM pulse/chow administration. Representative of n = 2 independent transplants. **p ≤ 0.01, ****p ≤ 0.0001. 

d. Kaplan–Meier survival analysis of MPN (CTRL) vs. tamoxifen (TAM; Jak2V617F-deleted) treated mice (n ≥ 12 per arm; Log-rank test). Gray bar represents duration of TAM pulse/chow administration. ****p ≤ 0.0001. 

e. Spleen weights of MPN (CTRL) vs. tamoxifen (TAM; Jak2V617F-deleted) treated mice at timed sacrifice (24 weeks) in comparison to wild-type control mice (mean ± s.e.m.). Representative of n = 2 independent transplants. ****p ≤ 0.0001. 

f. Heatmap demonstrating cytokine/chemokine concentrations (log10 concentration; pg/mL) in peripheral blood serum of MPN (CTRL) vs. tamoxifen (TAM; Jak2V617F-deleted) treated mice in comparison to wild-type control mice (n = 5 biological replicates per arm). 

g. Representative hematoxylin and eosin (H&E) and reticulin stains of bone marrow of MPN (Control) vs. tamoxifen (Jak2V617F-deleted) treated mice from timed sacrifice 24 weeks. Representative micrographs of n = 6 individual mouse replicates per arm. All images represented at 400X magnification. Scale bar: 20μm.
Figure 2

(a) PB chimerism

(b) % mutant cell fraction

(c) Hallmark pathway

(d) \(-\log_{10}(p)\) vs log2FoldChange

(e) % live lineage-neg cells

(f) CTRL, D3, D7

(g) % Target / % Background

72.8% / 18.03% 35.90% / 17.65%
Figure 2: *Jak2*V617F reversal impairs the fitness of MPN cells, including MPN stem cells. **a.** Peripheral blood (PB) mutant Cd45.2 percent chimerism trend (weeks 0-24) of early (3 weeks post-transplant) tamoxifen (TAM; *Jak2*V617F-deleted) treated (gold bar) and late (12 weeks post-transplant) tamoxifen treated (maroon bar) mice (n = 8 each) in comparison to MPN (CTRL; dark gray bar; n = 6) mice (mean ± s.e.m.). Gray bars represent duration of TAM pulse/chow administration. Representative of n = 2 independent transplants. **p ≤ 0.01, ***p ≤ 0.001. **b.** Bone marrow mutant cell fraction within LSK (Lineage*Sca1^+^cKit^+^), granulocytic-monocytic progenitor (GMP; Lineage^cKit^Sca1^Cd34^Fcg^-), and megakaryocytic-erythroid progenitor (MEP; Lineage^cKit^Sca1^Cd34^Fcg^-) compartments of early (3 weeks post-transplant) tamoxifen (TAM; *Jak2*V617F-deleted) treated and late (12 weeks post-transplant) tamoxifen treated mice in comparison to MPN (CTRL) mice at timed sacrifice 24 weeks (n = 6-8 individual biological replicates per arm; mean ± s.e.m.). Representative of n = 2 independent transplants. **p ≤ 0.01, ***p ≤ 0.001, ****p ≤ 0.0001. **c.** Gene-set enrichment analysis (GSEA) of significant Hallmark gene sets of MPN (CTRL) vs. tamoxifen (TAM; *Jak2*V617F-deleted) treated LSKs isolated 7 days following initiation of TAM (n = 3-4 biological replicates per arm). **d.** Volcano plot demonstrating differential gene expression of MPN (CTRL) vs. TAM (*Jak2*V617F-deleted) treated LSKs 7 days following initiation of TAM (n = 3-4 biological replicates per arm). **e.** GMP and MEP stem cell frequencies of MPN (CTRL) vs. tamoxifen (TAM; *Jak2*V617F-deleted) treated mice 7 days following initiation of TAM (n = 8 biological replicates per arm across 2 independent transplants; mean ± s.e.m.). **f.** Row normalized heatmap of RNA-sequencing data of key erythroid differentiation factor genes from harvested MEPs at baseline (CTRL), day 3 (D3) and day 7 (D7) following initiation of TAM (*Jak2*V617F deletion). **g.** HOMER motif analysis from ATAC-seq data demonstrating decreased accessibility of Gata motif signatures with concomitant increased accessibility of Cebp motif signatures of TAM treated (*Jak2*V617F-deleted) cKit^+^ bone marrow cells isolated 7 days following initiation of treatment in comparison to MPN (CTRL) cells (n = 3 biological replicates per arm).
Figure 3

(a) Log2FoldChange TAM vs. Control

(b) HEME_METABOLISM in LSK

(c) CTRL RUX CTRL TAM

(d) Human HSPCs

(e) WBC (Kul.)

(f) PB chimerism

(g) % mutant cell fraction

(h) KRAS DOWN in MEP

(i) Vehicle Ruxolitinib Tamoxifen

(j) Rel. Vav Exp. (Actr)
Figure 3: Differential efficacy of Jak2<sup>V617F</sup> deletion compared to JAK inhibitor therapy. 

**a.** Scatter plot depicting log2FoldChange of ruxolitinib (RUX) treated vs. tamoxifen (TAM; Jak2<sup>V617F</sup>-deleted) treated LSKs (Lineage<sup>-</sup>Sca1<sup>+</sup>cKit<sup>+</sup>) in comparison to MPN control LSKs isolated after 7 days of treatment (n = 2-3 biological replicates per arm); differentially expressed genes as indicated by color (see Supplemental Tables 1 and 3). 

**b.** Gene set enrichment analysis (GSEA) depicting a positive enrichment in heme metabolism in ruxolitinib (RUX) treated (n = 3) vs. negative enrichment in tamoxifen (TAM; Jak2<sup>V617F</sup>-deleted) treated (n = 3) LSKs isolated after 7 days of treatment. 

**c.** Row normalized heatmap of RNA-sequencing data of ruxolitinib (RUX) treated (blue) or tamoxifen (TAM; Jak2<sup>V617F</sup>-deleted) treated (red) megakaryocytic-erythroid progenitor (MEP; Lineage<sup>-</sup>cKit<sup>+</sup>Sca1<sup>-</sup>Cd34<sup>-</sup>Fcg<sup>-</sup>) cells in comparison to MPN (CTRL) cohorts (gray). 

**d.** Box plots of single-cell ATAC-seq motif accessibility for either NFkB1 or REL transcription factors for untreated human JAK2 wild-type (n = 188 cells from 4 patients; gray), untreated JAK2<sup>V617F</sup>-mutant (n = 105 cells from 4 patients; gray), and ruxolitinib-treated JAK2<sup>V617F</sup>-mutant (n = 87 cells from 3 patients; blue) HSPCs (from Myers, R.M. and Izzo, F. et al., bioRxiv, 2022). P values indicated are from linear mixture model explicitly modeling patient identity as random effect to account for patient-specific effects, followed by likelihood ratio test. ****p ≤ 0.0001. 

**e.** Peripheral blood counts of vehicle (VEH), ruxolitinib (RUX), or tamoxifen (TAM; Jak2<sup>V617F</sup>-deleted) treated mice at the conclusion of a 6-week <i>in vivo</i> trial: white blood cells (WBC; left panel), hematocrit (Hct; right panel) (n ≥ 4 each; mean ± s.e.m). **p ≤ 0.01, ***p ≤ 0.001, ****p ≤ 0.0001. 

**f.** Peripheral blood (PB) mutant Cd45.2 percent chimerism trend (0-6 weeks) of vehicle (VEH), ruxolitinib (RUX), or tamoxifen (TAM; Jak2<sup>V617F</sup>-deleted) treated mice (n ≥ 4 each; mean ± s.e.m.). *p ≤ 0.05. 

**g.** Bone marrow mutant cell fraction of LSK (Lineage<sup>-</sup>Sca1<sup>+</sup>cKit<sup>+</sup>), granulocytic-monocytic progenitor (GMP; Lineage<sup>-</sup>cKit<sup>+</sup>Sca1<sup>-</sup>Cd34<sup>-</sup>Fcg<sup>-</sup>), and megakaryocytic-erythroid progenitor (MEP; Lineage<sup>-</sup>cKit<sup>+</sup>Sca1<sup>-</sup>Cd34<sup>-</sup>Fcg<sup>-</sup>) compartments of vehicle (VEH), ruxolitinib (RUX), or tamoxifen (TAM; Jak2<sup>V617F</sup>-deleted) treated mice at the conclusion of the 6-week <i>in vivo</i> trial (n ≥ 4 each; mean ± s.e.m). *p ≤ 0.05, ****p ≤ 0.0001. 

**h.** GSEA depicting a negative enrichment in down-regulation of KRAS signaling targets in ruxolitinib (RUX) treated (n = 3) vs. positive enrichment in tamoxifen (TAM; Jak2<sup>V617F</sup>-deleted) treated (n = 3) MEPs isolated following 7 days of respective treatment. 

**i.** Immunohistochemistry of phospho-ERK on sectioned bone marrow of vehicle, ruxolitinib, or tamoxifen (Jak2<sup>V617F</sup>-deleted) treated mice following 7 days of treatment (n = 3 individual biological replicates per arm).
All images represented at 400X magnification. Scale bar: 20μm. j. Quantitative polymerase-chain reaction demonstrating relative $Ybx1$ expression levels from isolated cKit$^+$ bone marrow of vehicle (VEH) vs. ruxolitinib (RUX) vs. tamoxifen (TAM; Jak$^{2V617F}$-deleted) treated mice following 7 days of treatment ($n = 2-4$ individual biological replicates per arm; mean ± s.e.m). *$p < 0.05$, **$p \leq 0.01$. e-g. Representative of $n = 3$ independent experiments.
Figure 4

(a) Schematic diagram of the experimental setup. Jak2RL donor bone marrow cells were transplanted into Jak2RL/Tet2−/− recipients. After treatment with TAM, the spleen weight was measured.

(b) Graph showing the WBC counts (K/μL) over 21 weeks post-transplantation. The graph compares Jak2RL, Jak2RL/Tet2−/−, and Jak2RL/TAM groups.

(c) Graph showing the spleen weight (mg) over 21 weeks post-transplantation. The graph compares Jak2RL, Jak2RL/Tet2−/−, and Jak2RL/TAM groups.

(d) Graph showing the percentage of mutant cell fraction over 21 weeks post-transplantation. The graph compares Jak2RL, Jak2RL/Tet2−/−, and Jak2RL/TAM groups.

(e) Graphs showing the WBC and Hct levels over 21 weeks post-transplantation. The graphs compare Jak2RL, Jak2RL/Tet2−/−, and Jak2RL/TAM groups.

(f) Graph showing the PB chimerism over 21 weeks post-transplantation. The graph compares Jak2RL, Jak2RL/Tet2−/−, and Jak2RL/TAM groups.

(g) Micrographs showing the tissue samples from control and Tamoxifen-treated groups.

(h) Graph showing the percentage of mutant cell fraction in LSK cells from Jak2RL and Jak2RL/Tet2−/− groups. The graph compares CTRL and TAM-treated groups.

(i) Graph showing the number of colonies formed in the plating experiments. The graph compares Jak2RL, Jak2RL/TAM, Jak2RL/Tet2−/− CTRL, and Jak2RL/Tet2−/− TAM groups.
Figure 4: Jak2V617F dependency with cooperative Tet2 loss. a. Schematic of the experimental set up for the double-mutant Jak2RL/Tet2+/− competitive transplants. TAM: tamoxifen; KI: knock-in; KO: knock-out; Lin-neg BM: lineage-negative bone marrow; cGy: centigray. Downward arrows represent initial pulse TAM administration to genetically inactivate Tet2. b. White blood cell (WBC) counts of primary Jak2RL vs. Jak2RL/Tet2+/− transplanted mice at 16 weeks post-transplant (n = 5 each; mean ± s.d.). Representative of an n = 2 independent transplants. *p ≤ 0.05. c. Spleen weights of primary Jak2RL vs. Jak2RL/Tet2+/− transplanted mice at time of sacrifice (n = 5 each; mean ± s.d.). Representative of an n = 2 independent transplants. *p ≤ 0.05. d. Peripheral blood Cd45.2 mutant percent chimerism of Jak2RL vs. Jak2RL/Tet2+/− secondary competitive transplant mice at 16 weeks post-transplant (n = 7-8 each; mean ± s.d.). Representative of an n = 2 independent transplants. **p ≤ 0.01. e. Peripheral blood count trends (weeks 0-21) of MPN (CTRL) vs. tamoxifen (TAM; Jak2V617F-deleted) treated Jak2RL vs. Jak2RL/Tet2+/− competitive transplant mice: white blood cells (WBC; left panel), hematocrit (Hct; right panel) (n = 3-4 per arm; mean ± s.e.m). Gray bars represent duration of TAM pulse/chow administration. Representative of n = 2 independent transplants. **p ≤ 0.01, ***p ≤ 0.001, ****p ≤ 0.0001. f. Percent change in Cd45.2 mutant peripheral blood chimerism pre- vs. post-tamoxifen (Jak2V617F-deletion) treatment of Jak2RL vs. Jak2RL/Tet2+/− mice in relation to Cd45.1 competitor cells (n = 3-4 per arm; mean ± s.e.m). Representative of n = 2 independent transplants. g. Reticulin stains of bone marrow from MPN control vs. tamoxifen (Jak2V617F-deleted) treated Jak2RL vs. Jak2RL/Tet2+/− mice at timed sacrifice 21 weeks. Representative micrographs of n = 3 individual mouse replicates per arm. All images represented at 400X magnification. Scale bar: 20μm. h. Bone marrow mutant Cd45.2 percent chimerism within the LSK (Lin−Sca1+cKit+) compartment of MPN (CTRL) vs. tamoxifen (TAM; Jak2V617F-deleted) treated Jak2RL vs. Jak2RL/Tet2+/− mice at timed sacrifice 21 weeks (n ≥ 7 biological replicates per arm across 2 independent transplants; mean ± s.e.m). *p ≤ 0.05, ***p ≤ 0.001. i. Serial replating assay of plated MPN (CTRL) vs. tamoxifen (TAM; Jak2V617F-deleted) treated Jak2RL vs. Jak2RL/Tet2+/− bone marrow cells harvested at timed sacrifice 21 weeks and scored at day 8 after each plating (each sample plated in triplicate, representative of n = 2 independent experiments, mean ± s.d.).
Extended Data Figure 1: Phenotypic characterization and validation of Jak2RL activation in vivo. a. Sanger sequencing of the Jak2RL locus from genomic DNA of sorted Cre TdTomato+ reporter bone marrow cells following tamoxifen administration (Jak2RL + Cre) compared to the non-recombined Jak2RL locus (Jak2RL) demonstrating retainment of lox/rox sites despite prior Cre exposure. Representative of n = 3 individual biological replicates. b. Schematic of the Dre mRNA Jak2RL bone marrow electroporation transplant protocol. Lin-neg BM: lineage-negative bone marrow; cGy: centigray. c. Representative polymerase chain reaction (PCR) of genomic DNA isolated from peripheral blood mononuclear cells in primary transplant recipients following Dre mRNA electroporation (+Dre) confirming the presence of Jak2V617F knock-in bands (KI) in relation to the Jak2 wild-type (WT) allele in comparison to unrecombined (-Dre) Jak2RL cells. d. Peripheral blood counts of wild-type (WT) vs. Jak2RL knock-in mice in comparison to the previously published Cre-lox conditional Jak2V617F knock-in mouse model (Jak2Crelox) at timed sacrifice of 16 weeks: white blood cells (WBC; left panel), hematocrit (Hct; middle panel), platelets (Plt; right panel) (n = 5 per arm; mean ± s.d.). *p < 0.05, **p < 0.01, ****p < 0.0001. e. Ter119+ Cd71+ erythroid precursor fractions (left panel) and representative flow cytometry plots (right panel) from harvested spleen cells of Jak2RL knock-in mice in comparison to wild-type (WT) and the Jak2Crelox knock-in model (n = 5 per arm; mean ± s.e.m). Black boxes on flow plots denote Ter119+ erythroid progenitor stages I-IV. **p < 0.01. f. Bone marrow myeloid progenitor (Lineage− cKit+Sca1−) frequencies of Jak2RL knock-in mice in comparison to wild-type (WT) and the Jak2Crelox knock-in model (n = 5 per arm; mean ± s.e.m). **p < 0.01. g. Spleen weights (left panel) and representative spleens (right panel) of Jak2RL knock-in mice in comparison to wild-type (WT) and the Jak2Crelox knock-in model at timed sacrifice 16 weeks post-transplant (n = 5 per arm; mean ± s.d.). **p < 0.01, ***p < 0.001. h. Hematoxylin and eosin (H&E) stains of bone marrow and spleen of Jak2RL knock-in mice in comparison to wild-type controls at timed sacrifice 16 weeks post-transplant. Representative micrographs of n = 5 individual mouse replicates per arm. Bone marrow images represented at 400X magnification (scale bar 20μm); spleen images 100X (scale bar 100μm). d-h. Representative of an n = 3 independent transplants.
Extended Data Figure 2: Functional consequences of Jak2^{V617F} reversion ex vivo. a. Schematic of the bone marrow endothelial cell (BMEC) co-culture assay.\textsuperscript{16} Lin-neg BM: lineage-negative BM; EtOH: ethanol vehicle; 4-OHT: 4-hydroxy-tamoxifen. b. Representative Jak2^{V617F} knock-in and knock-out genotyping by polymerase chain reaction (PCR) of genomic DNA isolated from cultured bone marrow cells following Dre-mediated knock-in (+Dre) and/or subsequent Cre-mediated deletion (+Dre+Cre) in comparison to the unrecombined Jak2^{RL} allele (CTRL). Representative bands of \( n = 4 \) individual replicates per arm. c. Total cell output of cultured wild-type (gray) vs. Jak2^{RL} knock-in (maroon) cells in comparison to Cre-inducible Jak2^{V617F} knock-in (Jak2^{Crelox}; gold)\textsuperscript{8} cells following 7 days of culture over BMECs in the presence of increasing doses (0 nM - 1000 nM) of 4-hydroxy-tamoxifen (4-OHT) \((n = 3\) technical replicates each; mean \( \pm \) s.d.). ns: not significant, **\( p < 0.01 \), ****\( p < 0.0001 \). d. Total cell, e. Mac1^{"Gr1\textsuperscript{high}} mature neutrophil, f. Myeloid progenitor (Lineage^{cKit^{Sca1}}), and g. Megakaryocytic-erythroid progenitor (MEP; Lineage^{cKit^{Sca1}Cd34^{Fcg}}) cell output of Jak2^{RL} knock-in cells in comparison to wild-type vs. Cre-inducible Jak2^{Crelox} MPN cells following 7 days of culture over BMECs in the presence of vehicle (VEH) vs. 400 nM 4-hydroxy-tamoxifen (4-OHT) \((n = 3\) technical replicates each; mean \( \pm \) s.d.). ****\( p < 0.0001 \). h. Total erythroid precursor cell numbers by Ter119/Cd71 erythroid progenitor flow cytometry (I-IV; left panel) and representative Ter119/Cd71 flow plots (right panel) of cultured Jak2^{RL} MPN cells following 7 days of culture over BMECs in the presence of vehicle (VEH) vs. 400 nM 4-hydroxy-tamoxifen (4-OHT) vs. 250 nM ruxolitinib (RUX) \((n = 3\) technical replicates each; mean \( \pm \) s.d.). **\( p < 0.01 \), ****\( p < 0.0001 \), ****\( p < 0.0001 \). i. Percentages of Mac1^{"Gr1\textsuperscript{high}} Annexin V+ apoptotic cells of Jak2^{RL} cells in comparison to wild-type vs. Jak2^{Crelox} cells following 7 days of culture over BMECs in the presence of vehicle (VEH) vs. 200 nM 4-hydroxy-tamoxifen (4-OHT) \((n = 3\) technical replicates each; mean \( \pm \) s.d.). *\( p < 0.05 \), **\( p < 0.01 \). c-i. Representative of \( n = 2 \) independent experiments.

Extended Data Figure 3: Functional characterization of Jak2^{RL} knock-in/knock-out in vivo. a. Schematic of the experimental set up for the Jak2^{RL} knock-in/knock-out transplants. BM: bone marrow; TAM: tamoxifen; cGy: centigray. b. Representative polymerase chain reaction (PCR) demonstrating presence or loss of Jak2^{RL} knock-in (KI) bands in relation to the Jak2 wild-type (WT) allele from reporter-sorted whole bone marrow mononuclear cells based on different recombined states: Pre-Dre: unrecombined Jak2^{RL} cells; double-neg: reporter-negative cells
following Dre recombination; TdTomato+: *Jak2*<sup>V617F</sup> knock-in population following Dre recombination; GFP+: green fluorescent protein (GFP) population: *Jak2*<sup>V617F</sup>-deleted population (i.e. Dre followed by Cre). c. *Jak2*<sup>V617F</sup> mutant RNA transcript variant allele frequency (VAF) from isolated megakaryocytic-erythroid progenitor (MEP; Lineage<sup>cKit<SUP>+</SUP>Sca1<sup>−</sup>Cd34<sup>−</sup>Fcg<sup>−</sup>)) cells 7 days following tamoxifen (TAM; *Jak2*<sup>V617F</sup> deletion) treatment in comparison to MPN (CTRL) mice (n = 3-5 each; mean ± s.d.). **** *p* ≤ 0.0001. d. Platelet (Plt) counts of MPN (CTRL) vs. tamoxifen (TAM; *Jak2*<sup>V617F</sup>-deleted) treated mice at timed sacrifice 24 weeks (n ≥ 10 each; mean ± s.e.m). Representative of n = 2 independent transplants. *** *p* ≤ 0.001. e. Representative polymerase chain reaction (PCR) demonstrating presence of *Jak2*<sup>V617F</sup> knock-in (KI) bands in relation to the *Jak2* wild-type (WT) allele from isolated bone marrow mononuclear cells of transplant recipients previously treated with tamoxifen (Jak2<sup>V617F</sup> deletion) and exhibiting phenotypic features of recurrent MPN (n = 2) in comparison to those with no recurrent MPN (representative of n = 8 biological replicates). f. Representative hematoxylin and eosin (H&E) stains and Ter119 immunohistochemistry of sectioned spleen of MPN control vs. tamoxifen (Jak2<sup>V617F</sup>-deleted) treated mice at timed sacrifice 24 weeks. Representative micrographs of n = 6 individual mouse replicates per arm. All images represented at 100X magnification. Scale bar: 100μm.

**Extended Data Figure 4: Assessment of tamoxifen toxicity on *Jak2<sup>RL</sup>* knock-in cells. a.** Peripheral blood count trend (weeks 0-15) of MPN (CTRL) vs. tamoxifen (TAM) treated mice transplanted with Cre-negative *Jak2<sup>RL</sup>* knock-in cells in competition with Cd45.1 support: white blood cells (WBC; left panel), hematocrit (Hct; middle panel), platelets (PLT; right panel) (n = 3-4 each; mean ± s.e.m). Gray bars represent duration of TAM pulse/chow administration. ns = not significant, *p* ≤ 0.05. b. Peripheral blood (PB) mutant Cd45.2 percent chimerism trend (weeks 0-15) of MPN (CTRL) vs. tamoxifen (TAM) treated mice transplanted with Cre-negative *Jak2<sup>RL</sup>* knock-in cells in competition with Cd45.1 support (n = 3-4 each; mean ± s.e.m). Gray bar represents duration of TAM pulse/chow administration. c. Spleen weights (left panel) and representative isolated spleens (right panel) of MPN (CTRL) vs. tamoxifen (TAM) treated mice transplanted with Cre-negative *Jak2<sup>RL</sup>* knock-in cells in competition with Cd45.1 support at timed sacrifice 15 weeks (n = 3-4 each; mean ± s.e.m). d. Total bone marrow (BM) cellularity (femur) and e. Myeloid progenitor (Lineage<sup>cKit<sup>+</sup>Sca1<sup>+</sup>) frequencies of MPN (CTRL) vs. tamoxifen (TAM) treated mice transplanted with Cre-negative *Jak2<sup>RL</sup>* knock-in cells in competition with
Cd45.1 support at timed sacrifice 15 weeks (n = 3-4 each; mean ± s.e.m). f. Bone marrow mutant cell fraction within LSK (Lineage-Sca1-`cKit+), granulocytic-monocytic progenitor (GMP; Lineage`cKit+Sca1`Cd34`Fcg+), and megakaryocytic-erythroid progenitor (MEP; Lineage`cKit`Sca1`Cd34`Fcg+) compartments of MPN (CTRL) vs. tamoxifen (TAM) treated mice transplanted with Cre-negative \( \text{Jak2}^{\text{RL}} \) knock-in cells in competition with Cd45.1 support at timed sacrifice 15 weeks (n = 3-4 each; mean ± s.e.m). g. Hematoxylin and eosin (H&E) stains of bone marrow (BM) of MPN (CTRL) vs. tamoxifen (TAM) mice transplanted with Cre-negative \( \text{Jak2}^{\text{RL}} \) knock-in cells in competition with Cd45.1 support at timed sacrifice 15 weeks. Representative micrographs of n = 3-4 individual mouse replicates. Images represented at 400X magnification. Scale bar: 20μm. a-g. Representative of n = 3 independent experiments.

Extended Data Figure 5: Effects of \( \text{Jak2}^{V617F} \) deletion on MPN stem cell fitness and disease transplantability. a. Schematic of the experimental set up for the \( \text{Jak2}^{\text{RL}} \) competitive transplant studies. TAM: tamoxifen; cGy: centigray. b. Peripheral blood count trends (weeks 0-24) of early (3 weeks post-transplant) tamoxifen (TAM; \( \text{Jak2}^{V617F} \)-deleted) treated (gold) and late (12 weeks post-transplant) tamoxifen treated (maroon) mice in comparison to MPN (CTRL; gray) mice: white blood cells (WBC; left panel), hematocrit (Hct; right panel) (n = 6-8 per arm; mean ± s.e.m). Gray bars represent duration of TAM pulse/chow administration. Representative of n = 2 independent transplants. **p ≤ 0.01, ***p ≤ 0.001, ****p ≤ 0.0001. c. Peripheral blood Mac1`Gr1\text{high} mutant Cd45.2 percent chimerism trend (weeks 0-24) of early (3 weeks post-transplant) tamoxifen (TAM; \( \text{Jak2}^{V617F} \)-deleted) treated (gold) and late (12 weeks post-transplant) tamoxifen treated (maroon) mice in comparison to MPN (CTRL; gray) mice (n = 6-8 each, mean ± s.e.m.). Gray bars represent duration of TAM pulse/chow administration. Representative of n = 2 independent transplants. **p ≤ 0.01, ***p ≤ 0.001. d. Bone marrow mutant Cd45.2 percentage within the long-term hematopoietic stem cell (LT-HSC; Lineage`Sca1`cKit`Cd150`Cd48-) compartment of early (3 weeks post-transplant) tamoxifen (TAM; \( \text{Jak2}^{V617F} \)-deleted) treated and late (12 weeks post-transplant) tamoxifen treated mice in comparison to MPN (CTRL) mice at timed sacrifice 24 weeks (n = 6-8 each; mean ± s.e.m). Representative of n = 2 independent transplants. **p ≤ 0.01, ****p ≤ 0.0001. e. Representative flow cytometry plots demonstrating mutant Cd45.2 to competitor Cd45.1 percentage of gated LSK (Lineage`Sca1`cKit+) cells of MPN (CTRL) vs. tamoxifen (TAM; \( \text{Jak2}^{V617F} \)-deleted) treated mice.
at timed sacrifice 24 weeks. Representative of n = 6-8 biological replicates per arm.

f. Hematocrit (Hct) and g. Peripheral blood mutant Cd45.2 fraction of recipient mice transplanted with MPN (CTRL) vs. tamoxifen (TAM; Jak2V617F-deleted) treated unfractionated donor bone marrow cells at 12 weeks post-transplant (n = 5 each; mean ± s.e.m). Representative of n = 2 independent experiments. *p ≤ 0.05, **p ≤ 0.01.

h. Polymerase chain reaction (PCR) demonstrating presence of a Jak2V617F knock-in (KI) band in relation to the Jak2 wild-type (WT) allele in 1 of 5 recipients (red asterisk) transplanted with tamoxifen (TAM; Jak2V617F-deleted) treated donor cells demonstrating recurrence of MPN phenotype in comparison to donor mice transplanted with MPN (CTRL) bone marrow donor cells.

Extended Data Figure 6: Acute phenotypic and transcriptional changes following Jak2V617F reversion.

a. Heatmap (left panel) and gene set enrichment analysis (GSEA; right panel) demonstrating negative enrichment of STAT5 gene targets, including negative regulators of JAK/STAT signaling, in LSK (Lineage–Sca1+cKit+) cells harvested 3 days (D3) +/- 7 days (D7) following initiation of tamoxifen (Jak2V617F deletion) treatment in comparison to MPN (CTRL) cells.

b. GSEA demonstrating negative enrichment in KEGG:MAPK signaling targets from harvested LSKs 7 days following initiation of tamoxifen treatment in comparison to MPN control (CTRL) LSKs.

c. Mac1+ immunohistochemistry (IHC) of sectioned bone marrow (BM; left panel) and bone marrow Mac1+Gr1high frequencies by flow cytometry (right panel) of MPN (CTRL) vs. tamoxifen (TAM; Jak2V617F-deleted) treated mice at 7 days following treatment (n = 4 each; mean ± s.e.m.). Representative micrographs of n = 4 individual mouse replicates each. Images represented at 400X magnification. Scale bar: 20μm. **p ≤ 0.01.

d. Total erythroid progenitor bone marrow cell numbers, and e. Representative flow cytometry plots of Ter119/Cd71 erythroid progenitor populations of MPN (CTRL) vs. tamoxifen (TAM; Jak2V617F-deleted) treated mice 7 days following treatment (n = 4-7 per arm; mean ± s.e.m.). *p ≤ 0.05, **p ≤ 0.01, ***p ≤ 0.001.

f. Burst-forming unit erythroid (BFU-E) colony output of MPN (CTRL) vs. tamoxifen (TAM; Jak2V617F-deleted) treated marrow isolated 7 days following TAM administration and scored at day 8 after plating (each sample plated in triplicate, mean ± s.e.m.).

g. Normalized ATAC-Seq signal at the EpoR locus (top panel) with associated quantified normalized peak counts (bottom panel) demonstrating decreased accessibility in tamoxifen (TAM; Jak2V617F-deleted) treated cKit+
bone marrow cells 7 days following initiation of TAM in comparison to MPN (CTRL) cells. d-f.
Representative of \( n = 2 \) independent experiments.

**Extended Data Fig. 7: Differential responses of \( \text{Jak2}^{V617F} \) deletion compared to JAK inhibitor therapy in vivo.**

a. Normalized read counts of representative JAK/STAT targets from LSK (Lineage\( ^{−} \)Sca1\(^{+} \)cKit\(^{+} \)) cells isolated 7 days following ruxolitinib (RUX) vs. tamoxifen (TAM; \( \text{Jak2}^{V617F} \) deletion) treatment compared to MPN controls (CTRL) (\( n = 2-4 \) per arm; mean \pm \) s.e.m.).

b. LSK (Lineage\( ^{−} \)Sca1\(^{+} \)cKit\(^{+} \); left panel), granulocytic-monocytic progenitor (GMP; Lineage\( ^{−} \)cKit\(^{+} \)Sca1\(^{−} \)Cd34\(^{+} \)Fcg\(^{+} \); middle panel), and megakaryocytic-erythroid progenitor (MEP; Lineage\( ^{−} \)cKit\(^{+} \)Sca1\(^{−} \)Cd34\(^{−} \)Fcg\(^{−} \); right panel) bone marrow frequencies of vehicle (VEH), ruxolitinib (RUX), or tamoxifen (TAM; \( \text{Jak2}^{V617F} \)-deleted) treated mice 7 days following respective treatment (\( n = 3-5 \) per arm; mean \pm \) s.e.m.). Data representative of \( n = 2 \) independent experiments. \(*p \leq 0.05, ***p \leq 0.001, ****p \leq 0.0001.\)

c. Heatmap of single-cell ATAC-seq motif accessibilities for NF\( \kappa \)B transcription factors for untreated (\( n = 105 \) cells from 4 patients) and ruxolitinib treated (\( n = 87 \) cells from 3 patients) human \( \text{JAK2}^{V617F} \) mutant hematopoietic stem/progenitor cells (HSPCs) (from Myers, R. and Izzo, F. et al., bioRxiv, 2022).

d. Schematic of the competitive transplant set up for the \( \text{Jak2}^{RL} \) in vivo drug studies. cGy: centigray.

e. Platelet (PLT) counts and f. Spleen weights of vehicle (VEH), ruxolitinib (RUX), or tamoxifen (TAM; \( \text{Jak2}^{V617F} \)-deleted) treated mice at the conclusion of the 6-week in vivo trial (\( n \geq 4 \) each; mean \pm \) s.e.m). \(*p \leq 0.05, **p \leq 0.01, ***p \leq 0.001, ****p \leq 0.0001.\) Representative of \( n = 3 \) independent experiments.

g. Hematoxylin and eosin (H&E) stains of bone marrow and spleen sections from vehicle, ruxolitinib, or tamoxifen (\( \text{Jak2}^{V617F} \)-deleted) treated mice at the conclusion of the 6-week in vivo trial. Representative micrographs of \( n = 4 \) individual mouse replicates per arm. Bone marrow images represented at 400X magnification (scale bar 20\( \mu \)m); spleen images 100X (scale bar 100\( \mu \)m).

h. Box plots of single-cell ATAC-seq motif accessibility for FOS (left panel) and JUN (right panel) transcription factors for untreated (\( n = 188 \) cells from 4 patients; light gray) or ruxolitinib treated (\( n = 55 \) cells from 3 patients; light blue) \( \text{JAK2} \) wild-type human HSPCs in comparison to untreated (\( n = 105 \) cells from 4 patients; dark gray) or ruxolitinib-treated (\( n = 87 \) cells from 3 patients; dark blue) \( \text{JAK2}^{V617F} \)-mutant HSPCs (from Myers, R.M. and Izzo, F. et al., bioRxiv, 2022). \( P \) values indicated are from linear mixture model explicitly modeling patient identity as random effect to account for patient-specific effects, followed by likelihood ratio test. \(*p \leq 0.01, ****p \leq 0.0001.\)
Extended Data Fig. 8: Phenotypic characterization and Jak2<sup>V617F</sup> oncogenic dependency in the setting of concomitant Tet2 loss. 

**a.** Hematocrit (Hct; left panel) and platelet counts (Plt; right panel) of Jak2<sup>RL</sup> vs. Jak2<sup>RL</sup>/Tet2<sup>−/−</sup> primary transplanted mice at 16 weeks post-transplant (n = 5; mean ± s.d.). Representative of an n = 2 independent transplants. 

**b.** Serial replating assay of Jak2<sup>RL</sup> vs. Jak2<sup>RL</sup>/Tet2<sup>−/−</sup> bone marrow cells in relation to wild-type bone marrow. Colonies were counted at day 8 after each plating (each sample plated in triplicate, n = 2 independent experiments, mean ± s.d.).

**c.** Total Cd45.2 cell (left panel) and Mac1<sup>+</sup>Gr1<sup>high</sup> mature neutrophil cell (right panel) output of Jak2<sup>RL</sup> vs. Jak2<sup>RL</sup>/Tet2<sup>−/−</sup> cells following 7 days of culture over bone marrow endothelial cells (BMECs) (n = 3 replicates per arm; mean ± s.d.). Representative of n = 2 independent experiments. *p ≤ 0.05, **p ≤ 0.01.

**d.** Spleen weights (left panel) and representative isolated spleens (right panel) of MPN (CTRL) vs. tamoxifen (TAM; Jak2<sup>V617F</sup>-deleted) treated Jak2<sup>RL</sup> vs. Jak2<sup>RL</sup>/Tet2<sup>−/−</sup> mice (n ≥ 7 biological replicates per arm across 2 independent transplants; mean ± s.d.). *p ≤ 0.05, ****p ≤ 0.0001.

**e.** Total bone marrow (BM) cellularity (femur), and **f.** Bone marrow mutant cell fraction within the granulocytic-monocytic progenitor (GMP; Lineage<sup>−</sup>cKit<sup>+</sup>Sca1<sup>−</sup>Cd34<sup>+</sup>Fcg<sup>+</sup>; left panel) and megakaryocytic-erythroid progenitor (MEP; Lineage<sup>−</sup>cKit<sup>+</sup>Sca1<sup>−</sup>Cd34<sup>−</sup>Fcg<sup>−</sup>; right panel) compartments of MPN (CTRL) vs. tamoxifen (TAM; Jak2<sup>V617F</sup>-deleted) treated Jak2<sup>RL</sup> vs. Jak2<sup>RL</sup>/Tet2<sup>−/−</sup> mice (n ≥ 7 biological replicates per arm across 2 independent transplants; mean ± s.e.m.). *p ≤ 0.05, **p ≤ 0.01, ***p ≤ 0.001.

**g.** Representative Jak2<sup>V617F</sup> knock-in (left panel) and Tet2<sup>−/−</sup> excision genotyping (right panel) by polymerase chain reaction (PCR) of genomic DNA isolated from whole bone marrow of MPN (CTRL) vs. tamoxifen (TAM; Jak2<sup>V617F</sup>-deleted) treated Jak2<sup>RL</sup> vs. Jak2<sup>RL</sup>/Tet2<sup>−/−</sup> mice from time of sacrifice demonstrating presence or loss of Jak2<sup>V617F</sup> knock-in (KI) and/or Tet2<sup>−/−</sup> excised (knock-out; KO) bands in relation to respective wild-type (WT) bands. Representative bands of n = 4–7 individual replicates per arm across 2 independent transplants.
Extended Data Figure 1 – Phenotypic characterization and validation of \( \text{Jak}^{2\text{RL}} \) activation in vivo

**a**

<table>
<thead>
<tr>
<th>Lox2272 #1</th>
<th>Ros #1</th>
<th>Ros #2</th>
<th>Lox2272 #1</th>
</tr>
</thead>
<tbody>
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</table>

\( \text{Jak}^{2\text{RL}} \)

\( \text{Jak}^{2\text{RL}} + \text{Cre} \)

**b**

Dre mRNA electroporation

Lin-neg BM

Ubc:CreER- \( \text{Jak}^{2\text{RL}} \) donor

Cd45.2

(\( N=5/\text{donor} \))

**c**

\( \text{Dre}^+\text{Dre} \)

\( \text{Dre}^-\text{Dre} \)

**d**

<table>
<thead>
<tr>
<th>WBC (K/uL)</th>
<th>Hct (%)</th>
<th>Platelets (K/uL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild-type</td>
<td>Jak2(^{RL})</td>
<td>Wild-type</td>
</tr>
<tr>
<td>WT</td>
<td>Lox2272</td>
<td>WT</td>
</tr>
</tbody>
</table>

**e**

\( \% \text{Ter119}^+\text{CD71}^+ \text{live cells} \)

**f**

<table>
<thead>
<tr>
<th>% Ter119(^+)CD71(^+) live cells</th>
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</thead>
<tbody>
<tr>
<td>Wild-type</td>
</tr>
<tr>
<td>WT</td>
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</table>

**g**

<table>
<thead>
<tr>
<th>Spleen weight (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild-type</td>
</tr>
<tr>
<td>WT</td>
</tr>
</tbody>
</table>

**h**

Wild-type

Bone marrow

Spleen

Wild-type

\( \text{Jak}^{2\text{RL}} \)

Spleen
Extended Data Figure 2 – Functional consequences of Jak2V617F reversion ex vivo

(a) Ubc:CreER-Jak2RL donor (active MPN)

(b) Total cellularity

(c) Myeloid progenitor

(d) Mac1+Gr+

(e) Erythroid Progenitor Stage

(f) Total cellularity

(g) MEP

(h) Erythroid Progenitor Stage

(i) % Annexin positive cells
Extended Data Figure 3 – Functional characterization of Jak2RL knock-in/knock-out in vivo

**a** Clone 2 donors Ubc:CreER-Jak2RL (active MPN) → Whole BM → CD45.2 donor

**C**d45.2 donor

**b** Ubc:CreER-Jak2RL (active MPN)

**c** Transplant

**2nd** Transplant

12 weeks post-transplant:

TAM (Jak2V617F deletion)

(Cd45.1+GFP+TdTomato+) (N=10/arm)

Control

**Jak2RL KI**

**Jak2 WT**

**d** TGF (production)

**e** H&E

**f** Ter119

**g** Control

**h** Tamoxifen

**i** Recurrent MPN

**j** No MPN

**k** Whole BM was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

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Extended Data Figure 4 – Assessment of tamoxifen toxicity on Jak2<sup>RL</sup> knock-in cells

**a** BM cell count (femur) (x10<sup>6</sup>)

**b** % live lin-neg cells

**c** Spleen weight (mg)

**d** % 45.2 reporter chimerism

**e** % mutant cell fraction

**f** % mutant cell fraction

**g** BM H&E
Extended Data Figure 5 – Effects of Jak2<sup>V617F</sup> deletion on MPN stem cell fitness and disease transplantability

(a) Competitive Transplant

Cd45.1 donor

Cd45.2 donor

Mac1<sup>+</sup>Gr1<sup>+</sup> high

Cd45.2 chimerism

% mutant cell fraction

Weeks post-transplant

CTRL Early TAM Late TAM

(b) WBC and Hematocrit

WBC (K/uL)

Hct (%)

Weeks post-transplant

CTRL Early TAM Late TAM

(c) LT-HSC

Lin<sup>-</sup>Sca1<sup>+</sup>cKit<sup>+</sup>

Sca1<sup>-</sup>PECy7<sup>-</sup>cKit<sup>-</sup>BV785<sup>-</sup>

Cd45.2-BUV395

(d) CD45.2 chimerism

CTRL Early TAM Late TAM

(e) Spleen weight

Spleen weight (mg)

CTRL Early TAM Late TAM

(f) Hct (%)

CTRL TAM

% mutant cell fraction

CTRL TAM

(g) % mutant cell fraction

CTRL TAM

% mutant cell fraction

CTRL TAM
Extended Data Figure 6 – Acute phenotypic and transcriptional changes following \textit{Jak2}^{V617F} reversion

\textbf{a} JAK/STAT5

\textbf{b} KEGG:MAPK Signaling Down in D7 LSK vs CTRL LSK

\textbf{c} Control vs Tamoxifen

\textbf{d} Total BFU-E

\textbf{e} Analysis of hematopoietic cell populations

\textbf{f} Normalized count under peak

\textbf{g} EpoR expression
Extended Data Figure 7 – Differential responses of Jak2\textsuperscript{V617F} deletion compared to JAK inhibitor therapy in vivo

a) 

b) 

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a) 

b)
Extended Data Figure 8 – Phenotypic characterization and Jak2^{V617F} oncogenic dependency in the setting of concomitant Tet2 loss.